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Bacteriological and physiochemical evaluation of locally produced tiger nut drink (kunu aya) sold in Owerri metropolis Imo state Nigeria

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Abstract

The public heath implication of consumption of contaminated and unregulated hawked drinks has received great attention in recent times. This work investigated succession parameters of micro-organisms and physiochemical characteristics of tiger nut (*Cyperus esculentus*) drink hawked in Owerri metropolis over a 72 hour period. Samples of freshly produced drinks were purchased from seven major locations (Ekeonuwa, Control, Ikenegbu, Futo campus, Ihiagwa, Obinze and Naze junction) in Owerri metropolis. Microbial evaluation of the drink samples was carried out using spread plate method. Physiochemical analysis of tiger nut drinks were carried out using standard A.O.A.C methods. The results indicated the presence of *Staphyloccus* sp, *Micrococcus* sp, *Enterobacter* sp, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella* sp, *Escherichia coli* and *Corynebacterium sp*. These contrasted from organisms isolated from laboratory prepared control. The mean total bacterial counts indicated tiger nut drink sold around Naze recorded the highest total heterotrophic bacterial count (10^9 CFU/ml), tiger nut drinks sold at FUTO campus, Obinze and Ihiagwa recorded the highest total fecal coliform count (10^4 CFU/ml) and total *Salmonella-Shigella* count (10^4 CFU/ml), while the laboratory prepared recorded the least heterotrophic bacterial count (10^3 CFU/ml), zero total fecal coliform count and total *Salmonella-Shigella* count recorded. The pH and titratable acidity over a 72 hour period indicated that all the drinks were acidic, where the street sold drinks recorded the highest acidity compared to the laboratory prepared drink. Very low microbial count observed for the laboratory prepared tiger nut drinks underscores the importance of Good Manufacturing Practices (GMP). It is recommended that regulatory government agencies for food and drink products should step up campaign for adoption/implementation of GMP by local vendors/producers.

Keyword: Tiger nut drink; Physiochemical properties; Microbial load; GMP; Bacteriological

1. Introduction

Tiger nut drink is a locally-produced beverage, which is commonly consumed by many individuals for its beneficial purposes such as prevention of heart problems, thrombosis, can enhance weight loss, reduces colon cancer risk and as a dairy substitute having the presence of beneficial probiotics [1]. These attributes owe to its high energy, mineral and vitamin contents. This high nutritional profile contributes to its high contamination rate leading to short shelf life <12hours [2].

The contamination of the product can result from procedures employed in preparation and handling of the drink from the point of material collection to packaging. These stages are undertaken by vendors who may use low grade raw materials. Some may use unsanitized and unsterilized processing equipment (blender/grinder) and recycled bottles [3]. These unregulated practices has been of great public health concern, as a good number of people consume this product hawked on the streets by individuals without permission from appropriate food authorities [4].

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The production negligence of some producers and lack of supervision from the food regulating authorities in some parts of Africa, Nigeria inclusive, has made it difficult to ascertain the level of implication of this drink in food poisoning outbreaks.

The objective of this project is to determine the bacteriological and physiochemical analysis of tiger nut drinks sold in Owerri metropolis, Imo State.

2. Material and methods

2.1. Sample Collection

A total of 70 freshly prepared samples were randomly obtained from different tiger nut sellers around FUTO campus, Ihiagwa market, Obinze junction, Ekeonuwa market, Control, Ikenegbu and Naze junction, all in Owerri Metropolis, South-east, Nigeria. The samples were placed in ice-packed coolers and transported to the laboratory immediately for analysis.

2.2. Preparations of Tiger nut drink (Kunu Aya)

Tiger nuts were sorted out to remove broken and rotten nuts, stones, pebbles, and other dirt materials before rinsing in water to remove adhering soils. Other ingredients used in the milk preparation (coconut, date palm) were properly washed before use. The shell of the coconut was cracked and removed using knife and the water discarded, the coconut endosperm was cut into smaller pieces. The seed of the date are removed, discarded and the entire raw materials were thoroughly washed in warm water. One kilogramme (1 kg) of tiger nuts was soaked in3 liters of boiled water at 60 °C for 6 hours, drained and blanched at 70 °C for 5 mins, mainly to inactivate enzymes that might cause clumping of the extract. The tiger nuts were blended with 300 g of coconut and 150 g of date in 6 L of cooled boiled water several times into slurry with sterilized blender and slurry pressed using muslin cloth to extract the milk [5].

2.3. Bacteriological Analysis

Ten milliliters of fresh tiger nut drink were dispersed and swirled thoroughly in 90 ml of freshly prepared peptone water respectively. Ten fold dilutions were carried out serially on the mixtures. Quantities of 10^{-6} dilutions were evenly inoculated using spread plate method in duplicate into Nutrient agar plates and for other selective media (10^{-3}) respectively. The five media used for recovery of isolates included Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA), and *Salmonella-Shigella* Agar (SSA). The plates were incubated at 37 °C for 24hrs. Plates having colonies were counted using the Neubauer Colony Counter and counts recorded as colony forming units (CFU) per milliliters [6].

2.4. Determination of pH and titratable acidity of kunu aya

The pH of the samples was measured using a pH meter model radiometer. Total acidity was determined on a 10-ml sample by titrating with 0.1N NaOH to an endpoint, using 3drops of phenolphthalein as indicator. The percent titratable acidity was determined as described by A. O. A. C. [7].

The mean of TTA was obtained from a triplicate determination and calculated as follows:

$$TTA\% = \frac{AVERAGE\ TITRE\ VALUE\ X\ 0.1M\ X\ 0.009008}{WEIGHT\ OF\ SAMPLE} X \frac{100}{1}$$

2.5. Statistical Analysis

Data obtained from the analysis were subjected to analysis of variance (ANOVA).

3. Results

3.1. Determination of Bacterial isolates from the Tiger nut drink samples

The predominance of isolates recovered from the drink samples is shown in figure 1. A total of 226 isolates were recovered. Results revealed presence of *Staphyloccus* sp (15.9 %), *Micrococcus* sp (9.3 %), *Enterococci* sp (13.7 %), *Bacillus subtilis* (10.2 %), *Bacillus cereus* (5.8 %), *Salmonella* sp (5.3 %), *Escherichia coli* (7.1 %) and *Corynebacterium sp* (9.7 %) for the street sold tiger nut drink samples, while the laboratory prepared tiger nut drink samples recorded *Staphyloccus*

sp (2.7 %), *Micrococus* sp (2.7 %) , *Bacillus subtilis* (1.3 %), *Bacillus cereus* (1.3 %), and the absence of *Enterobacter* sp, *Salmonella* sp, *Escherichia coli* and *Corynebacterium sp*.

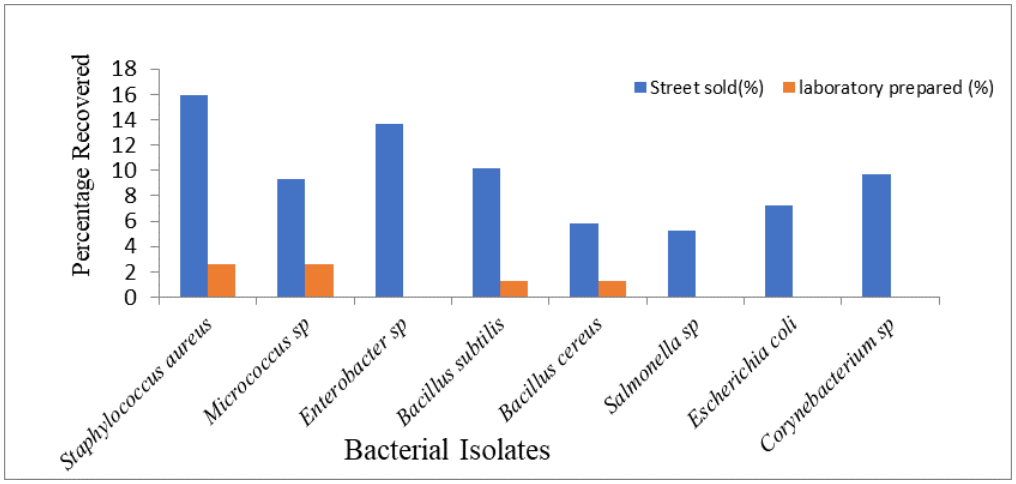
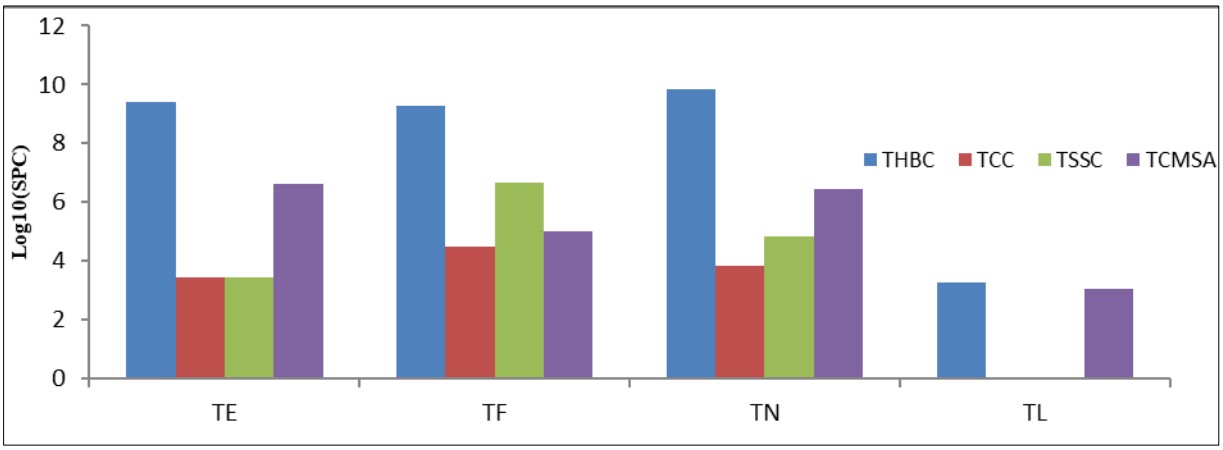


Figure 1 Predominance of isolates recovered from tiger nut drinks

3.2. Mean Total Viable Counts of Samples

Figure 2 shows the mean total viable counts of the tiger nut drink samples which identified Total heterotrophic bacterial counts (THBC), Total fecal coliform count (TCC), Total *Salmonella*-Shigella counts (TSSC) and Total Staphylococcus counts (TCMSA) using their logarithm values respectively. It was reported that THBC recorded the highest count for the tiger nut drinks sold in Naze junction (TN) (10^9 CFU\ml), the drinks sold around FUTO campus, Ihiagwa and Obinze (TF) recorded the highest TCC (10^4 CFU\ml) and TSSC (10^6 CFU\ml), while tiger nut drinks sold around Ekeonuwa, Ikenegbu and Control (TE) recorded the highest TCMSA (10^6 CFU\ml). The laboratory prepared drink sample (TL) recorded the least mean total viable counts of (10^3 CFU\ml) for THBC and TCMSA respectively and no TCC and TSSC recorded.



Key note: TN= Naze junction,TF= FUTO campus, Ihiagwa and Obinze,TE= Ekeonuwa, Ikenegbu and Control and TL= laboratory prepared drink sample

Figure 2 Mean Total Viable Counts of tiger nut drink

3.3. Titratable acidity and pH of samples during storage

Tables 1 and 2 show the pH and titratable acidity of the samples under storage which reported that all Tiger nut drink samples were acidic, where lower pH ranging from 4.9 to 4.5 and the higher acidity ranging from 0.0604 % to 0.0721 % at the time of production was recorded for streets sold drinks, and observed to steadily reduce (3.6 to 3.1) as well as increase (0.1126 to 0.1351 %) for pH and titratable acidity respectively. The Laboratory prepared drinks treated, untreated and pasteurized tiger nut drink samples recorded pH of 6.8 to 6.5 and titratable acidity of 0.0090 to 0.0144 % which steadily reduced to a pH of 5.1 to 5.0 and TTA steadily increased from 0.0252 to 0.0703 % under 72 hours storage time.

3.4. Total Counts (CFU\ml) of Bacterial Isolates in Drinks during Storage

Table 3 shows the total bacterial count on the tiger nut drink samples which reported that the street sold tiger nut drinks has the highest bacterial counts ranging from $1.7x10^8$ to $3.7x10^8$ CFU/ml while The Laboratory prepared drinks (treated, untreated and pasteurized) had bacterial count from $2.3x10^3$ to $2.5x10^3$ CFU/ml at the time of production and steady increase of microbial growth was observed during storage 72hrs ranging from $6.0x10^{11}$ to $2.9x10^{12}$ CFU/ml and $1.08x10^5$ to $7.1 x10^7$ CFU/ml for street sold drinks and laboratory prepared drink samples respectively. The laboratory prepared tiger nut drinks recorded the least microbial load with the pasteurized laboratory drink sample having no growth at the time of production.

Table 1 pH of the Tiger nut drinks during storage

Sample code	0hr	3hrs	6hrs	9hrs	12hrs	24hrs	36hrs	48hrs	60hrs	72hrs
TE	4.9±0.14	4.4±0.14	4.7±0.07	4.7±0.21	4.3±0.28	4.2±0.31	3.8±0.12	3.3±0.2	3.2±0.1	3.1±0.1
TF	4.8±0. 05	4.1±0. 02	3.9±0. 1	3.5± 0. 1	3± 0.03	3.8±0. 21	3.6±0. 21	3.9±0.07	3.2±0.14	3.1±0.07
TN	4.5± 0.05	4.9±0.01	4.5± 0.1	4.1±0.07	4± 02	3.6± 0.03	3.5± 0.14	3.5± 0.1	3.6 ±0. 01	3.6± 0.1
CN	6.5±0.12	6.4±0.01	6.9±0.01	6.7±0.02	6.4±0.14	6.1±0.2	5.9±0.1	5.8±0.21	5.3±0.02	5.1±0.1
CP	6.8±0.1	6.7±0.07	6.5±0.0.2	5.8±0.14	5.6±0.07	5.6±0.14	5.6±0.42	5.4.±0.2	5.4±0.1	5.0±0.14

key: TE= Tiger nut drink bought from Ekeonuwa, Control and Ikenegbu , TF=Tigernut drink bought from FUTO campus, Ihiagwa and Obinze TN= Tigernut drink bought from Nazi junction, Cn=Tiger nut drink without pasteurization, Cp =Pasteurized Tiger nut drink

Table 2 Titratable acidity (%) of Tiger nut drinks during storage

Sample code	0hr	3hrs	6hrs	9hrs	12hrs	24hrs	36hrs	48hrs	60hrs	72hrs
TE	0.0604	0.0784	0.0811	0.0901	0.0910	0.0937	0.0973	0.1027	0.1072	0.1126
TF	0.0631	0.0802	0.0892	0.0919	0.0973	0.0991	0.1009	0.1171	0.1189	0.1306
TN	0.0721	0.0793	0.0847	0.0955	0.1054	0.1135	0.1225	0.1270	0.1333	0.1351
CN	0.0144	0.0117	0.0126	0.0213	0.0216	0.0234	0.0234	0.0279	0.0306	0.0333
CP	0.0091	0.0180	0.0127	0.0143	0.0179	0.0206	0.0222	0.0226	0.0226	0.0233

key: TE= Tiger nut drink bought from Ekeonuwa, Control and Ikenegbu , TF=Tigernut drink bought from FUTO campus, Ihiagwa and Obinze TN= Tigernut drink bought from Nazi junction, Cn=Tiger nut drink without pasteurization, Cp =Pasteurized Tiger nut drink

Table 3 Total Counts (CFU\ml) of bacterial isolates in Tiger nut drinks during storage

Sample code	0hr	3hrs	6hrs	9hrs	12hrs	24hrs	36hrs	48hrs	60hrs	72hrs
TE	$2.8x10^8$	$6.8x10^8$	$1.7x10^9$	$2.1x10^9$	$2.9x10^{10}$	$3.9x10^{10}$	$1.7x10^{10}$	$2.5x10^{11}$	$6.5x10^{11}$	$8.4x10^{11}$
TF	$1.7x10^8$	$5.9x10^8$	$7.3x10^8$	$8.8x10^9$	$2.5x10^{10}$	$2.8x10^{10}$	$5.0x10^{11}$	$7.6x10^{11}$	$8.1x10^{11}$	$2.9x10^{12}$
TN	$3.7x10^8$	$4.5x10^8$	$5.1x10^8$	$6.3x10^9$	$6.8x10^9$	$1.2x10^{10}$	$2.8x10^{10}$	$2.9x10^{11}$	$4.4x10^{11}$	$6.0x10^{11}$
CN	$2.5x10^3$	$7.1x10^3$	$1.1x10^3$	$2.7x10^4$	$8.4 x10^4$	$3.7 x10^5$	$7.6 x10^5$	$8.3 x10^6$	$9x10^6$	$7.1 x10^7$
CP	-	$1.2x10^3$	$2.3x10^3$	$5.6x10^3$	$3.9x 10^4$	$1.09x10^5$	$2.5x10^6$	$4.8x10^6$	$8.5x10^6$	$3.4x10^7$

key: TE= Tiger nut drink bought from Ekeonuwa, Control and Ikenegbu , TF=Tigernut drink bought from FUTO campus, Ihiagwa and Obinze TN= Tigernut drink bought from Nazi junction, Cn=Tiger nut drink without pasteurization, Cp =Pasteurized Tiger nut drink

4. Discussion

This study bacteriological study was carried out, which showed a high microbial load in the street-sold tiger nut drinks in Owerri Metropolis with isolates such as *Staphyloccus* sp (15.9 %), *Micrоccus* sp (9.3 %), *Enterobacter* sp (13.7 %), *Bacillus subtilis* (10.2 %), *Bacillus cereus* (5.8 %), *Salmonella* sp (5.3 %), *Escherichia coli* (7.2 %) and *Corynebacterium sp* (9.7 %) is similar to the findings of Ibrahim et al., [5] in Sokoto, Nigeria, Olofu, Adeshina, and Olayinka [8] and Francis et al., [9] in Rivers state, Nigeria, this is a pointer to the fact that tiger nut drink sold in some parts of Owerri, majorly this specific selling points, are contaminated with bacteria, probably as a result of bad water used for preparation, equipment in grinding the ingredients which are market heavy-duty grinders that are highly cross-contaminated, local producers' poor hygiene and poorly sterilized or unsterilized recycled bottles for packaging as well as the ingredients not properly handled, leading to high microbial contamination, while *Staphyloccus* sp (2.7 %), *Micrоccus* sp (2.7 %) , *Bacillus subtilis* (1.3 %), *Bacillus cereus* (1.3 %), and the absence of *Enterobacter* sp, *Salmonella* sp, *Escherichia coli* and *Corynebacterium sp*. recorded for the laboratory prepared tiger nut drink samples. The presence of *Enterococci* sp, *E coli* or *Salmonella* sp in the street sold samples is an indicator of fecal organisms belonging to the family *Enterobacter*iaceae which are common inhabitants of the intestinal tract of humans and animals and contamination of the drink as studies have shown could be a result of poor sanitation, personal hygiene of the producers and the poor sewage drainage system [10].

The other bacterial isolates such as *Staphylococcus aureus* and *Bacillus* species are known to be associated with foodborne intoxication through the production of enterotoxin, *Staphylococcus aureus* is a normal flora of the skin that could be introduced into the drink product from the skin of humans when handling the products and packaging the packaging of the drink studies have shown that the enterotoxin produced by *Staphylococcus* and some coagulase-negative *Staphylococcus* which affect the central nervous system are also heat stable [11]. *Bacillus* species have been reported to be a causative agent of contamination in the milk and dairy industry [12]. The others, *Corynebacterium sp* and *Micrococci* sp recorded, might be due to improper cleaning and blanching procedure of the ingredients which interact with environmental factors such as soil water and dust, as it has been reported to be a natural habitat for *Micrococcus* sp. *Corynebacterium sp* isolated from the sample is of great public health which has been isolated from soil, animal feces, fruits and vegetable and has been traced to contaminate foods that have high interaction level with the soil and animal feces in the field, which has been linked to food spoilage and contamination as well as infection in immune compromised patients [9]. This report has shown that the Tiger nut drink sold in Owerri streets is not suitable for human consumption as the microbial load at the time of production to the stated storage time was above 5.0 logs10 CFU/ml according to Microbiological Guidelines for Food [13], which stated that the total aerobic count of less the 10^5 CFU/ml is satisfactory for consumption, $10^5 \leq 10^7$ CFU/ml is a borderline for consumption and greater than 10^7 CFU/ml is unsatisfactory for human consumption. The laboratory-prepared tiger nut drink was reported to have a lower microbial load with only lower microbial counts of *Staphylococcus aureus*, *Micrococci* sp, and *Bacillus* sp and the microbial load from the time of production to the stated storage time for 72hours (10^3 to 10^7 CFU/ml) met the guideline for ready to eat food as safe according to the above stated guideline.

The pH and titratable acidity (TTA) of the tiger nut drink samples were studied which recorded lowest pH of 4.9 to 4.5 and TTA recorded the highest of 0.0604 to 0.0721 % at the point of preparation for street sold tiger nut drinks while pH between 6.8 and 6.5 and TTA of 0.0091 to 0.0144 % at the point of production for the laboratory prepared tiger nut drink was recorded. At the end of the stated 72 hours storage time, it was observed that the street sold tiger nut drinks recorded a reduced pH of 3.6 to 3.1 and an increased TTA of 0.1126 to 0.1351 % while the laboratory prepared tiger nut drinks recorded 5.1 to 5.0 and TTA of 0.0233 to 0.0333 %. The high level of acidity in the samples sold in Owerri is similar to study carried out by Adesakin, and Obiekezie [10] in Nasarawa, Nigeria and Musa, and Hamza [14] in Kaduna, which could be attributed to the presence of fermenting organisms such as lactic acid bacteria and can also mean that the drinks have started undergoing spoilage even before they were taken to the selling sites, resulting to production of metabolites and thus reduction of pH in the tiger nut drinks [14].

5. Conclusion

In conclusion, this research has shown the presence and high total bacterial count particularly enteric pathogens isolated from streets sold tiger nut drinks in Owerri metropolis compared to laboratory prepared drinks, it is also noted that the pasteurized tiger nut drink at the zero hour recorded no microbial growth. Implementation of Good Manufacturing Practices which is totally neglected by local producers can reduce the microbial load, especially the enteric pathogens which can bring about food poison and intoxication in tiger nut products when consumed.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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